IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner: C. BROWN; Art Unit: 1616; Docket No.: 1951

In RE: **Application of Susanne KESSLER, et al**

Ser. No.: **10/030,278**

Filing Date: **April 9, 2002**

AUGUST 14, 2008

SECOND DECLARATION OF FACTS FILED UNDER 37 C.F.R. 1.132

TO OVERCOME A REJECTION UNDER 35 U.S.C. 103 (a)

Hon. Commissioner of Patents

and Trademarks,

Washington, D.C. 20231

Sir:

In response to the final Office Action dated December 20, 2007 and in addition to the accompanying simultaneous amendment filed under 37 C.F.R. 1.114, please accept the following showing of experimental facts supporting the claims of the above-identified U.S. Patent Application:

WHEREAS WE, Susanne **KESSLER** and Sean **LEE**, citizens of Germany, whose post office addresses and residences are, respectively, Johannisweg 23, 84030 Ergolding, Germany; and Oberlinstrasse 17, 76227 Karlsruhe, Germany; have applied for Letters Patent for a new and improved

PRESERVATIVE FOR PERISHABLE PREPARATIONS PARTICULARLY FOR COSMETIC AND PHARMACEUTICAL FORMULATIONS

in a U.S. Patent Application, Ser. No. 10/030,278, filed April 9, 2002, of which claims 36 to 46 were rejected under 35 U.S.C. 103 (a) in a final Official Action dated December 20, 2007 over US 5,290,544, issued to Shimono, et al, on March 1, 1994, in view of WO 98/11853 of Greenspan, published March 26, 1998, and further in view of US 5,762,950, issued to Yli-Urpo, et al, on June 9, 1998.

WHEREAS WE have tested aqueous compositions containing 1 percent by weight of bioactive glass particles of respective different particle sizes (d_{50}) from 4 to 30 μm as well as coarse bioactive glass particles with sizes from 150 to 600 μm and have found that bioactive glass particles with particle sizes (d_{50}) ≤ 10 μm, especially of 4 μm, are unexpectedly and surprisingly more effective in preventing growth of bacteria over a time period of several weeks.

WHEREAS WE have tested aqueous compositions containing 1 percent by weight of bioactive glass particles of respective different particle sizes (d_{50}) from 4 to 30 μm as well as coarse bioactive glass particles with sizes from 150 to 600

μm and have found that coarse grained bioactive glass particles with particle sizes (d_{50}) for from 150 to 600 μm do not inhibit growth of *E. Coli* over a time period of about one week.

WHEREAS WE have tested aqueous compositions containing 1 percent by weight of bioactive glass particles of respective different particle sizes (d_{50}) from 4 to 30 μm as well as coarse bioactive glass particles with sizes from 150 to 600 μm and have found that only bioactive glass particles with particle sizes (d_{50}) of about 4 μm completely prevent growth of *C. Albicans* (a yeast) and are effective against *A.Niger.*

I. TEST METHODS

Suspensions of bioactive glass particles of different particle sizes or size ranges in aqueous media were prepared. Each different aqueous suspension contained 1 % by weight of bioactive glass particles of a correspondingly different particle size (d_{50}) or size range. The different particle sizes (d_{50}) of the bioactive glass particles used in the tested aqueous suspensions were 4 μm, 10 μm, and 30 μm. One group of tested suspensions contained coarse bioactive glass particles with particle sizes (d_{50}) of from 150 to 600 μm.

These aqueous suspensions were tested for their susceptibility to contamination by microorganisms by methods according to Ph. Eur. 6^{th} Edition (med.). Each different aqueous suspension was inoculated at the start of the test

with a known common microorganism. The microorganisms that were introduced to the test solutions were *Escherichia Coli* (ATCC 8739), *Corynebacterium Xerosis* (ATCC 7711), *Staphylococcus aureus* (ATCC 6538), *Candidia albicans* (ATCC 10231), and *Aspergillus Niger* (ATCC 18404). The numbers in parenthesis designate the strain of the bacteria or other microbe tested. Samples of these strains are on deposit at the American Type Culture Collection (ATCC). During the testing the amounts of the respective microbes present at certain fixed time intervals of 48 hours, 7d, 14d, 21d, and 28d from the initial inoculation were measured in KBE/ml by standard test methods [Ph. Eur., 6^{th} Edition (med.)].

The bioactive glass particles had a standard chemical composition, designated MD01, which is a preferred composition for bioactive glass used in the present application according to claims 37 and 43 in the accompanying amendment dated August 14, 2008. The MD01 composition is given in Table I.

TABLE I. BIOACTIVE GLASS COMPOSITION

	MD01
SiO_2	45.00
Na_2O	24.00
CaO	24.50
P_2O_5	6.00
Al_2O_3	-
MgO	- .

II. TEST RESULTS

The following tables II to VI show the initial amount of the respective microbes in the different aqueous suspensions with the different sized bioactive glass particulates and the resulting amounts of the microbes after 48 hours, 7d, 14d, 21d, and 28d .

TABLE II: EFFECT OF BIOACTIVE GLASS OF VARIOUS PARTICLE SIZE ON THE GROWTH OF *E. Coli* IN AQUEOUS MEDIA (amounts in KBE/ml)

Particle Size (d_{50})	Initial	48h	7d	14d	21d	28d
4 µm	350,000	0	0	0	0	0
10 µm	350,000	0	0	0	0	0
30 µm	350,000	0	0	0	0	0
150-600 µm	350,000	4,800	20,000	230,000	140,000	170,000

TABLE III: EFFECT OF BIOACTIVE GLASS OF VARIOUS PARTICLE SIZE ON THE GROWTH OF *C. Xerosis* IN AQUEOUS MEDIA (amounts in KBE/ml)

Particle Size (d_{50})	Initial	48h	7d	14d	21d	28d
4 µm	230,000	0	0	0	0	0
10 µm	230,000	0	0	0	0	0
30 µm	230,000	0	0	0	0	0
150-600 µm	230,000	0	0	0	0	0

TABLE IV: EFFECT OF BIOACTIVE GLASS OF VARIOUS PARTICLE SIZE ON THE GROWTH OF *S. Aureus* IN AQUEOUS MEDIA (amounts in KBE/ml)

Particle Size (d_{50})	Initial	48h	7d	14d	21d	28d
4 µm	230,000	0	0	0	0	0
10 µm	230,000	< 100	0	0	0	0
30 µm	230,000	200	0	0	0	0
150-600 µm	230,000	< 100	0	0	0	0

TABLE V: EFFECT OF BIOACTIVE GLASS OF VARIOUS PARTICLE SIZE ON THE GROWTH OF *C. Albicans* IN AQUEOUS MEDIA (amounts in KBE/ml)

Particle Size (d_{50})	Initial	48h	7d	14d	21d	28d
4 µm	330,000	0	0	0	0	0
10 µm	330,000	15,000	< 100	0	0	0
30 µm	330,000	9,600	0	0	0	0
150-600 µm	330,000	140,000	35,000	20,000	7,600	12,000

TABLE VI: EFFECT OF BIOACTIVE GLASS OF VARIOUS PARTICLE SIZE ON THE GROWTH OF *A. Niger* IN AQUEOUS MEDIA (amounts in KBE/ml)

Particle Size (d_{50})	Initial	48h	7d	14d	21d	28d
4 µm	290,000	< 100	300	< 100	< 100	< 100
10 µm	290,000	210,000	320,000	190,000	130,000	130,000
30 µm	290,000	210,000	250,000	210,000	150,000	150,000
150-600 µm	290,000	170,000	340,000	170,000	140,000	150,000

III. CONCLUSIONS

Generally the effectiveness of the bioactive glass particulates in preventing bacterial contamination decreases with increasing grain or particle size.

The results in Tables II to VI show that the effectiveness of a bioactive glass particulate with a composition as claimed in applicants' claims 37 and 43 and with a grain size or particle size of 4 μm exceeds the specifications for an A criterion of the European Pharmacopeia, 3rd Edition, for topical products.

While the bioactive glass particulate with a grain size or particle size of 10 μm provides good prevention of bacterial contamination for the *E. Coli, C. Xerosis,* and *S. Aureus*, it is only effective in eliminating *C. Albicans*, a yeast, after seven days. In the case of *A. Niger* the 10-μm bioactive glass particulate is ineffective.

The coarse grained bioactive glass particulate with a particle size range from 150 to 600 μm was effective in prevent contamination in the case of only two of the five microbes tested. It is completely ineffective against *E. Coli* and *A. Niger* and only serves to reduce amounts of *C. Albicans*.

The surprising results of these tests lead one to conclude that bioactive glass particulates should have a particle size that is less than or equal to 10 μm, preferably 4 μm, in order to be effective in prevention of contamination of an aqueous cosmetic composition by a wide variety of different microbes. While the

4 µm bioactive glass particulate fulfills, and even exceeds, the A criteria of European Pharmacopeia, 3rd Edition, for topical products, the 10 µm bioactive glass particulate does not show this high quality effectiveness. However the 10 µm bioactive glass particulate is effective against four out of the five tested microorganisms.

Also it is unexpected that the coarse grained bioactive glass particulate is only effective against some bacteria and is not effective against *E. Coli*.

WE HEREBY DECLARE AND AFFIRM THAT ALL STATEMENTS made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statement may jeopardize the validity of the above-named application, any patent issuing thereon or any patent to which this Declaration is directed.

_____	_____
DATE	Susanne KESSLER
_____	_____
DATE	Sean LEE